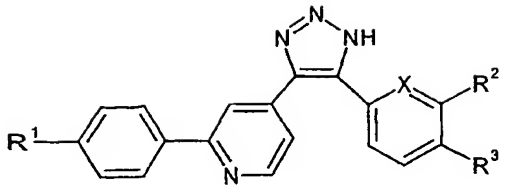
PYRIDINYL SUBSTITUTED (1,2,3) TRIAZOLES AS INHIBITORS OF THE TGF-BETA SIGNALLING PATHWAY

This invention relates to novel triazole derivatives which are inhibitors of the transforming growth factor, ("TGF")-ß signalling pathway, in particular, the phosphorylation of smad2 or smad3 by the TGF-β type I or activin-like kinase ("ALK")-5 receptor, methods for their preparation and their use in medicine, specifically in the treatment and prevention of a disease state mediated by this pathway.

TGF-β1 is the prototypic member of a family of cytokines including the TGF-βs, activins, inhibins, bone morphogenetic proteins and Müllerian-inhibiting substance, that signal through a family of single transmembrane serine/threonine kinase receptors. These receptors can be divided into two classes, the type I or activin like kinase (ALK) receptors and type II receptors. The ALK receptors are distinguished from the type II receptors in that the ALK receptors (a) lack the serine/threonine rich intracellular tail, (b) possess serine/threonine kinase domains that are very homologous between type I receptors, and (c) share a common sequence motif called the GS domain, consisting of a region rich in glycine and serine residues. The GS domain is at the amino terminal end of the intracellular kinase domain and is critical for activation by the type II receptor. Several studies have shown that TGF-β signalling requires both the ALK and type II receptors. Specifically, the type II receptor phosphorylates the GS domain of the type I receptor for TGF-β, ALK5, in the presence of TGF-β. The ALK5, in turn, phosphorylates the cytoplasmic proteins smad2 and smad3 at two carboxy terminal serines. The phosphorylated smad proteins translocate into the nucleus and activate genes that contribute to the production of extracellular matrix. Therefore, preferred compounds of this invention are selective in that they inhibit the type I receptor and thus matrix production.

Surprisingly, it has now been discovered that a class of novel triazole derivatives function as potent and selective non-peptide inhibitors of ALK5 kinase.

According to a first aspect, the invention provides a compound of formula (I), a pharmaceutically acceptable salt, solvate or derivative thereof:



(I)

wherein X is N or CH;

R^1 is selected from hydrogen, C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkoxy, halo, cyano, perfluoroC_{1-6}alkyl, perfluoroC_{1-6}alkoxy, $-NR^4R^5$, $-(CH_2)_nNR^4R^5$, $-O(CH_2)_nOR^6$, $-O(CH_2)_nNR^4R^5$, $-CONR^4R^5$, $-CO(CH_2)_nNR^4R^5$, $-SO_2R^6$, $-SO_2NR^4R^5$, $-NR^5SO_2R^6$ and $-NR^4COR^6$;

R^2 is hydrogen, C_{1-6}alkyl, halo, cyano or perfluoroC_{1-6}alkyl;

R^3 is hydrogen or halo;

R^4 and R^5 are independently hydrogen, C_{1-6}alkyl or Het; or R^4 and R^5 together with the nitrogen atom to which they are attached form a 3, 4, 5, 6 or 7-membered saturated or unsaturated ring which may contain one or more heteroatoms selected from N, S or O, and wherein the ring may be further substituted by one or more substituents selected from halo (such as fluoro, chloro, bromo), cyano, $-CF_3$, hydroxy, $-OCF_3$, C_{1-6}alkyl and C_{1-6}alkoxy;

R^6 is hydrogen or C_{1-6}alkyl;

Het is a 5 or 6-membered C-linked heterocyclyl group which may be saturated, unsaturated or aromatic, which may contain one or more heteroatoms selected from N, S or O and which may be substituted by C_{1-6}alkyl; and

n is 1-4.

The term "C_{1-6}alkyl" as used herein, whether on its own or as part of a group, refers to a straight or branched chain saturated aliphatic hydrocarbon radical of 1 to 6 carbon atoms, unless the chain length is limited thereto, including, but not limited to methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, pentyl and hexyl.

The term "alkenyl" as a group or part of a group refers to a straight or branched chain mono- or poly-unsaturated aliphatic hydrocarbon radical containing the specified number(s) of carbon atoms. References to "alkenyl" groups include groups which may be in the E- or Z-form or mixtures thereof.

The term "alkoxy" as a group or part of a group refers to an alkyl ether radical, wherein the term "alkyl" is defined above. Such alkoxy groups in particular include methoxy, ethoxy, n-propoxy, *iso*-propoxy, n-butoxy, *iso*-butoxy, *sec*-butoxy and *tert*-butoxy.

The term "perfluoroalkyl" as used herein includes compounds such as trifluoromethyl.

The term "perfluoroalkoxy" as used herein includes compounds such as trifluoromethoxy.

The terms "halo" or "halogen" are used interchangeably herein to mean radicals derived from the elements chlorine, fluorine, iodine and bromine.

The term "heterocyclyl" as used herein includes cyclic groups containing 5 to 7 ring-atoms up to 4 of which may be hetero-atoms such as nitrogen, oxygen and sulfur, and may be saturated, unsaturated or aromatic. Examples of heterocyclyl groups are furyl, thienyl, pyrrolyl, pyrrolinyl, pyrrolidinyl, imidazolyl, dioxolanyl, oxazolyl, thiazolyl, imidazolyl, imidazolinyl, imidazolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, pyranyl, pyridyl, piperidinyl, dioxanyl, morpholino, dithianyl, thiomorpholino, pyridazinyl, pyrimidinyl, pyrazinyl, piperazinyl, sulfolanyl, tetrazolyl, triazinyl, azepinyl, oxazepinyl, thiazepinyl, diazepinyl and thiazolinyl. In addition, the term heterocyclyl includes fused heterocyclyl groups, for example benzimidazolyl, benzoxazolyl, imidazopyridinyl, benzoxazinyl, benzothiazinyl, oxazolopyridinyl, benzofuranyl, quinolinyl, quinazolinyl, quinoxalinyl, dihydroquinazolinyl, benzothiazolyl, phthalimido, benzofuranyl, benzodiazepinyl, indolyl and isoindolyl.

Preferably X is N.

Preferably R^1 is C_{1-6}alkyl, C_{1-6}alkoxy, halo, perfluoroC_{1-6}alkoxy, $-(CH_2)_nNR^4R^5$, $-O(CH_2)_nNR^4R^5$, $-CONR^4R^5$ or $-SO_2R^6$. More preferably R^1 is C_{1-6}alkoxy, $-O(CH_2)_nNR^4R^5$ or $-CONR^4R^5$.

Preferably R^2 is hydrogen, C_{1-6}alkyl, chloro or fluoro. More preferably R^2 is hydrogen, methyl, chloro or fluoro. More preferably still R^2 is methyl.

Preferably R^3 is hydrogen or fluoro.

Preferably, when X is N, R^2 is methyl. More preferably when X is N and R^2 is methyl, R^3 is hydrogen.

Preferably R^4 and R^5 are independently hydrogen, C_{1-6}alkyl or Het; or R^4 and R^5 together with the nitrogen atom to which they are attached form a morpholine, piperidine, pyrrolidine, piperazine or N-methyl piperazine ring, each of which may be substituted by halo (such as fluoro, chloro, bromo), cyano, $-CF_3$, hydroxy, $-OCF_3$, C_{1-4}alkyl or C_{1-4}alkoxy.

More preferably R^4 and R^5 are independently hydrogen, C_{1-6}alkyl or tetrahydropyranyl; or R^4 and R^5 together with the nitrogen atom to which they are attached form a morpholine or pyrrolidine ring, each of which may be substituted by halo (such as fluoro, chloro, bromo), cyano, $-CF_3$, hydroxy, $-OCF_3$, C_{1-4}alkyl or C_{1-4}alkoxy.

It will be appreciated that the present invention is intended to include compounds having any combination of the preferred groups listed hereinbefore.

Preferably

X is N;

R^1 is C_{1-6}alkyl, C_{1-6}alkoxy, halo, perfluoroC_{1-6}alkoxy, $-(CH_2)_nNR^4R^5$, $-O(CH_2)_nNR^4R^5$, $-CONR^4R^5$ or $-SO_2R^6$;

R^2 is hydrogen, C_{1-6}alkyl, chloro or fluoro;

R^3 is hydrogen or halo;

R^4 and R^5 are independently hydrogen, C_{1-6}alkyl or Het; or R^4 and R^5 together with the atom to which they are attached form a morpholine, piperidine, pyrrolidine, piperazine or N-methyl piperazine ring, each of which may be substituted by halo (such as fluoro, chloro, bromo), cyano, $-CF_3$, hydroxy, $-OCF_3$, C_{1-4}alkyl or C_{1-4}alkoxy.

R^6 is hydrogen or C_{1-6}alkyl;

Het is a 5 or 6-membered C-linked heterocyclyl group which may be saturated, unsaturated or aromatic, which may contain one or more heteroatoms selected from N, S or O (preferably tetrahydropyranyl) and which may be substituted by C_{1-6}alkyl; and

n is 1-4.

Compounds of formula (I) which are of special interest as agents useful in the treatment or prophylaxis of disorders characterised by the overexpression of TGF-β are selected from the list:

2-(4-methanesulfonylphenyl)-4-(5-(6-methyl)-pyridin-2-yl-3H-[1,2,3]triazol-4-yl)-pyridine (Example 1);

2-(4-methoxyphenyl)-4-(5-(6-methyl)-pyridin-2-yl-3H-[1,2,3]triazol-4-yl)-pyridine (Example 2);

dimethyl-[2-(4-{4-[5-(6-methyl)-pyridin-2-yl-3H-[1,2,3]triazol-4-yl]-pyridin-2-yl}-phenoxy)-ethyl]-amine (Example 3);

4-(4-{4-[5-(6-methyl-pyridin-2-yl)-3H-[1,2,3]triazol-4-yl]-pyridin-2-yl}- benzyl)-morpholine (Example 4);

2-(4-ethylphenyl)-4-(5-(6-methyl-pyridin-2-yl)-3H-[1,2,3]triazol-4-yl)-pyridine (Example 5);

4-{4-[5-(6-methyl-pyridin-2-yl)-3H-[1,2,3]triazol-4-yl]-pyridin-2-yl}-*N*-(tetrahydro-pyran-4-yl)-benzamide (Example 6);

2-(4-chlorophenyl)-4-(5-(6-methyl)-pyridin-2-yl-3H-[1,2,3]triazol-4-yl)-pyridine (Example 7);

2-(4-trifluoromethoxyphenyl)-4-(5-(6-methyl)-pyridin-2-yl-3H-[1,2,3]triazol-4-yl)-pyridine (Example 8);

2-{4-(2-pyrrolidin-1-yl-ethoxy)-phenyl}-4-(5-(6-methyl)-pyridin-2-yl-3H-[1,2,3]triazol-4-yl)-pyridine (Example 9); and

2-(4-fluorophenyl)-4-(5-(6-methyl)-pyridin-2-yl-3H-[1,2,3]triazol-4-yl)-pyridine (Example 10);

and pharmaceutically acceptable salts, solvates and derivatives thereof.

For the avoidance of doubt, unless otherwise indicated, the term substituted means substituted by one or more defined groups. In the case where groups may be selected from a number of alternative groups, the selected groups may be the same or different.

For the avoidance of doubt, the term independently means that where more than one substituent is selected from a number of possible substituents, those substituents may be the same or different.

As used herein the term "pharmaceutically acceptable derivative" means any pharmaceutically acceptable salt, solvate, ester or amide, or salt or solvate of such ester or amide, of the compound of formula (I), or any other compound which upon administration to the recipient is capable of providing (directly or indirectly) the a compound of formula (I) or an active metabolite or residue thereof, e.g., a prodrug. Preferred pharmaceutically acceptable derivatives according to the invention are any pharmaceutically acceptable salts, solvates or prodrugs.

Suitable pharmaceutically acceptable salts of the compounds of formula (I) include acid salts, for example sodium, potassium, calcium, magnesium and tetraalkylammonium and the like, or mono- or di- basic salts with the appropriate acid for example organic carboxylic acids such as acetic, lactic, tartaric, malic, isethionic, lactobionic and succinic acids; organic sulfonic acids such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids and inorganic acids such as hydrochloric, sulfuric, phosphoric and sulfamic acids and the like. Some of the compounds of this invention may be crystallised or recrystallised from solvents such as aqueous and organic solvents. In such cases solvates may be formed. This invention includes within its scope stoichiometric solvates including hydrates as well as compounds containing variable amounts of water that may be produced by processes such as lyophilisation.

Hereinafter, compounds, their pharmaceutically acceptable salts, their solvates and polymorphs, defined in any aspect of the invention (except intermediate compounds in chemical processes) are referred to as "compounds of the invention".

The compounds of the invention may exist in one or more tautomeric forms. All tautomers and mixtures thereof are included in the scope of the present invention.

Compounds of the invention may exist in the form of optical isomers, e.g. diastereoisomers and mixtures of isomers in all ratios, e.g. racemic mixtures. The invention includes all such forms, in particular the pure isomeric forms. The different isomeric forms may be separated or resolved one from the other by conventional methods, or any given isomer may be obtained by conventional synthetic methods or by stereospecific or asymmetric syntheses.

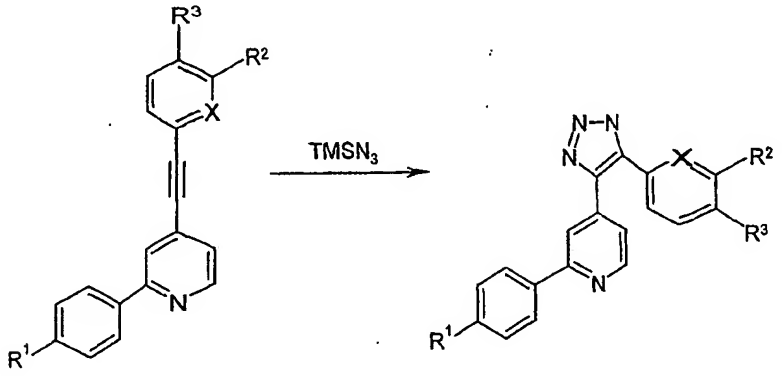
Since the compounds of the invention are intended for use in pharmaceutical compositions it will readily be understood that they are each preferably provided in substantially pure form, for example at least 60% pure, more suitably at least 75% pure and preferably at least 85%, especially at least 98% pure (% are on a weight for weight basis). Impure preparations of the compounds may be used for preparing the more pure forms used in the pharmaceutical compositions; these less pure preparations of the compounds should contain at least 1%, more suitably at least 5% and preferably from 10 to 59% of a compound of the invention.

Compounds of the invention may be prepared, in known manner in a variety of ways. In the following reaction schemes and hereafter, unless otherwise stated R^1 to R^6, X and n are as defined in the first aspect. These processes form further aspects of the invention.

Throughout the specification, general formulae are designated by Roman numerals (I), (II), (III), (IV) etc. Subsets of these general formulae are defined as (Ia), (Ib), (Ic) etc .... (IVa), (IVb), (IVc) etc.

Compounds of formula (I) may be prepared from compounds of formula (II) by treatment with an azide source according to reaction scheme 1. Preferred reaction conditions comprise treating compounds of formula (II) with trimethylsilylazide at elevated temperature in a suitable solvent such as dimethylformamide.

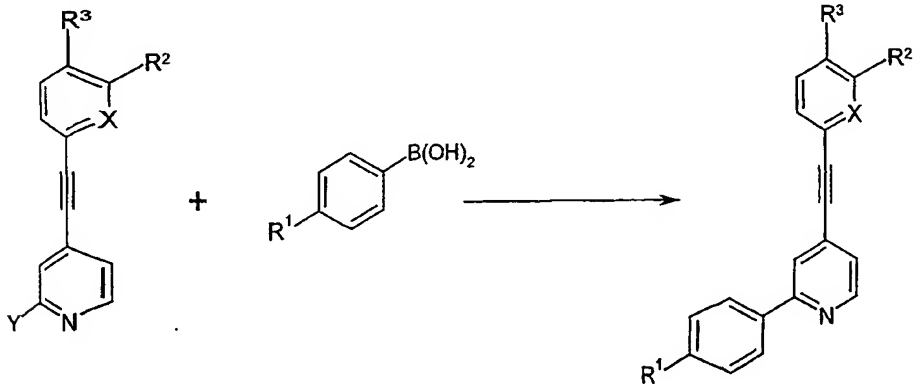
Scheme 1



(II) (I)

Compounds of formula (II) may be prepared by reacting compounds of formula (III) (where Y is a leaving group such as halogen preferably chlorine) with boronic acid derivatives of formula (IV) according to reaction scheme 2. Preferred conditions are those developed by Miyaura et al (Chem.Rev. 1995, 95: 2457), typically comprising reaction inert solvent in the presence of a base and a palladium or nickel catalyst at a temperature of between room temperature and 130°C for a period between 30 minutes and 48 hours. Suitable bases include sodium carbonate, potassium carbonate, potassium hydroxide, sodium hydroxide. Suitable catalysts include tetrakis(triphenlyphosphine) palladium(0), palladium(II) acetate, dichlorobis(triphenylphosphine) palladium(II), tris(dibenylideneacetone) dipalladium(0) and dichlorobis(triphenylphosphine) nickel.

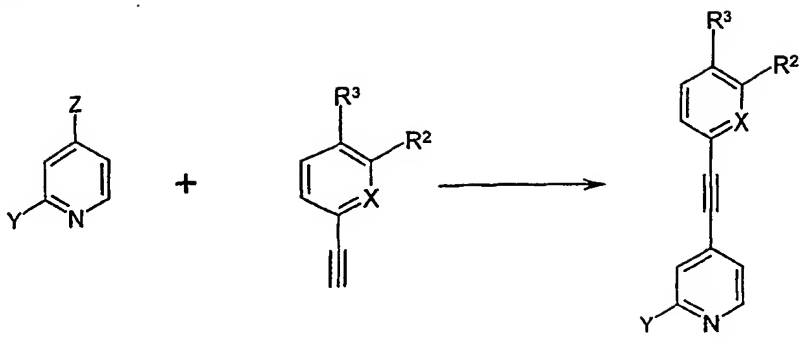
Scheme 2



(III) (IV) (II)

Compounds of formula (III) may be prepared by Sonagashira coupling of compounds of formula (V) (where preferably Y is chlorine and Z is iodine) with compounds of formula (VI) according to reaction scheme 3. Preferred reaction conditions comprise reaction in an inert solvent in the presence of a base and a palladium catalyst at a temperature of between room temperature and 80°C, for a period of between 30 minutes and 48 hours. Suitable bases include TMEDA or triethyl amine. Suitable palladium catalysts include tetrakis(triphenlyphosphine) palladium(0) and dichlorobis(triphenylphosphine) palladium(II).

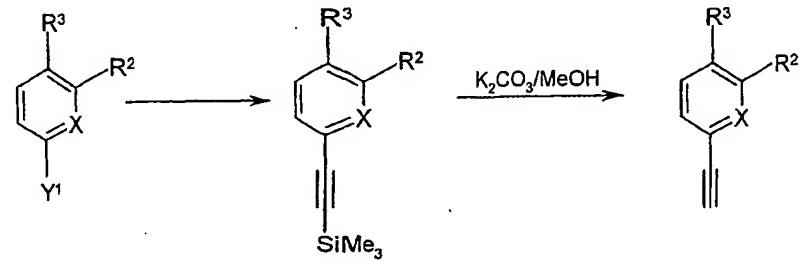
Scheme 3



(V) (VI) (III)

Compounds of formula (VI) may be prepared according to reaction scheme 4 where Y^1 in compounds of formula (VII) is a leaving group, preferably bromine. Preferred reaction conditions for the preparation of compounds of formula (VIII) comprise treating compounds of formula (VII) with trimethylsilylacetylene in the presence of TMEDA and copper iodide under palladium catalysis in an inert solvent such as tetrahydrofuran at elevated temperature. The trimethylsilyl group may be removed by treating compounds of formula (VIII) with a base such as potassium carbonate in a protic solvent such as methanol.

Scheme 4

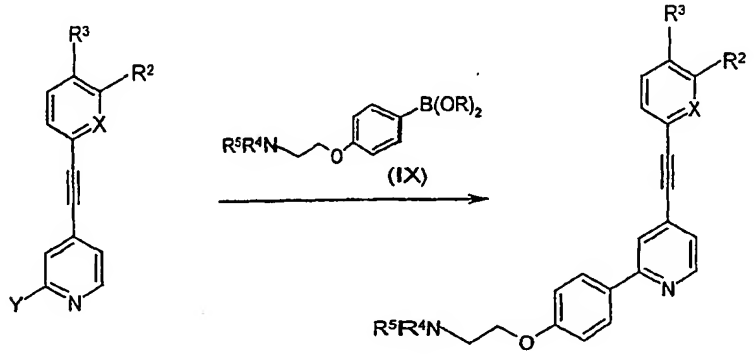


(VII) (VIII) (VI)

Compounds of formula (IIa), i.e. compounds of formula (II) where R^1 is $-O(CH_2)_2NR^4R^5$, may be prepared from compounds of formula (III) (where Y is preferably chlorine) according to reaction scheme 5. Compounds of formula (III) may be reacted with compounds of formula (IX) to give compounds of formula (IIa) in one step. Alternatively compounds of formula (III) may firstly be reacted with 4-hydroxy-

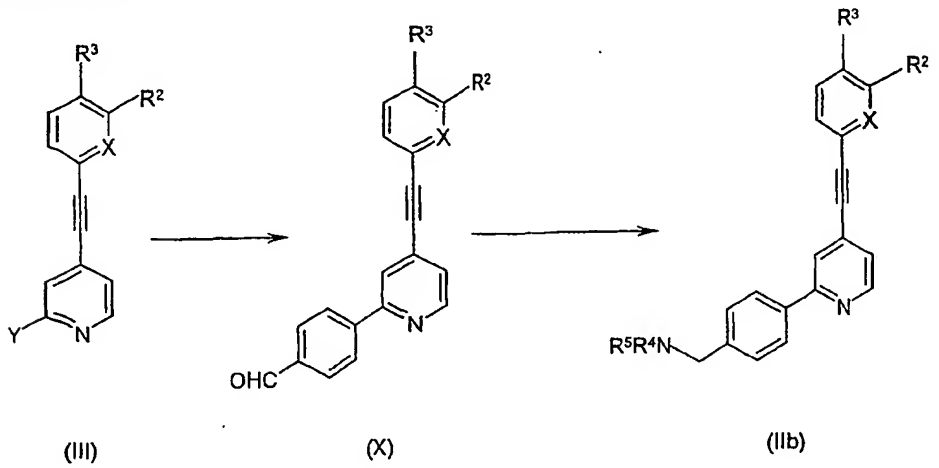
phenyl boronic acid, followed by alkylation with $R^4R^5N(CH_2)_2Cl$ in the presence of a base such as potassium carbonate or sodium hydride in a solvent such as dimethylformamide.

Scheme 5



Compounds of formula (IIb), i.e. compounds of general formula (II) where R^1 is $-CH_2NR^4R^5$, may be prepared according to reaction scheme 6. Compounds of formula (III) (where Y is preferably chlorine) may be reacted with 4-formylphenyl boronic acid using analogous conditions to reaction scheme 2 to give compounds of formula (X). Compounds of formula (X) may then be reacted with R^5R^4NH in the presence of a reducing agent, such as sodium cyanoborohydride in acetic acid at room temperature, to give compounds of formula (IIb).

Scheme 6



Further details for the preparation of compounds of formula (I) are found in the examples section hereinafter.

The compounds of the invention may be prepared singly or as compound libraries comprising at least 2, for example 5 to 1,000 compounds, and more preferably 10 to 100 compounds. Libraries of compounds of the invention may be prepared by a combinatorial 'split and mix' approach or by multiple parallel synthesis using either solution phase or solid phase chemistry, by procedures known to those skilled in the art. Thus according to a further aspect there is provided a compound library comprising at least 2 compounds of the invention.

Activation of the TGF-β1 axis and expansion of extracellular matrix are early and persistent contributors to the development and progression of chronic renal disease and vascular disease. Border W.A., *et al*, *N. Engl. J. Med.*, 1994; 331(19), 1286-92. Further, TGF-β1 plays a role in the formation of fibronectin and plasminogen activator inhibitor-1, components of sclerotic deposits, through the action of smad3 phosphorylation by the TGF-β1 receptor ALK5. Zhang Y., *et al*, *Nature*, 1998; 394(6696), 909-13; Usui T., *et al*, *Invest. Ophthalmol. Vis. Sci.*, 1998; 39(11), 1981-9.

Progressive fibrosis in the kidney and cardiovascular system is a major cause of suffering and death and an important contributor to the cost of health care. TGF-β1 has been implicated in many renal fibrotic disorders. Border W.A., *et al*, *N. Engl. J. Med.*, 1994; 331(19), 1286-92. TGF-β1 is elevated in acute and chronic glomerulonephritis Yoshioka K., *et al*, *Lab. Invest.*, 1993; 68(2), 154-63, diabetic nephropathy Yamamoto, T., *et al*, 1993, *PNAS* 90, 1814-1818., allograft rejection, HIV nephropathy and angiotensin-induced nephropathy Border W.A., *et al*, *N. Engl. J. Med.*, 1994; 331(19), 1286-92. In these diseases the levels of TGF-β1 expression coincide with the production of extracellular matrix. Three lines of evidence suggest a causal relationship between TGF-β1 and the production of matrix. First, normal glomeruli, mesangial cells and non-renal cells can be induced to produce extracellular-matrix protein and inhibit protease activity by exogenous TGF-β1 in vitro. Second, neutralizing anti-bodies against TGF-β1 can prevent the accumulation of extracellular matrix in nephritic rats. Third, TGF-β1 transgenic mice or in vivo transfection of the TGF-β1 gene into normal rat kidneys resulted in the rapid development of glomerulosclerosis. Kopp J.B., *et al*, *Lab. Invest.*, 1996; 74(6), 991-

1003. Thus, inhibition of TGF-β1 activity is indicated as a therapeutic intervention in chronic renal disease.

TGF-β1 and its receptors are increased in injured blood vessels and are indicated in neointima formation following balloon angioplasty Saltis J., *et al*, *Clin. Exp. Pharmacol. Physiol.*, 1996; 23(3), 193-200. In addition TGF-β1 is a potent stimulator of smooth muscle cell ("SMC") migration in vitro and migration of SMC in the arterial wall is a contributing factor in the pathogenesis of atherosclerosis and restenosis. Moreover, in multivariate analysis of the endothelial cell products against total cholesterol, TGF-β receptor ALK5 correlated with total cholesterol ($P < 0.001$) Blann A.D., *et al*, *Atherosclerosis*, 1996; 120(1-2), 221-6. Furthermore, SMC derived from human atherosclerotic lesions have an increased ALK5/TGF-β type II receptor ratio. Because TGF-β1 is over-expressed in fibroproliferative vascular lesions, receptor-variant cells would be allowed to grow in a slow, but uncontrolled fashion, while overproducing extracellular matrix components McCaffrey T.A., *et al*, Jr., *J. Clin. Invest.*, 1995; 96(6), 2667-75. TGF-β1 was immunolocalized to non-foamy macrophages in atherosclerotic lesions where active matrix synthesis occurs, suggesting that non-foamy macrophages may participate in modulating matrix gene expression in atherosclerotic remodelling via a TGF-β-dependent mechanism. Therefore, inhibiting the action of TGF-β1 on ALK5 is also indicated in atherosclerosis and restenosis.

TGF-β is also indicated in wound repair. Neutralizing antibodies to TGF-β1 have been used in a number of models to illustrate that inhibition of TGF-β1 signalling is beneficial in restoring function after injury by limiting excessive scar formation during the healing process. For example, neutralizing antibodies to TGF-β1 and TGF-β2 reduced scar formation and improved the cytoarchitecture of the neodermis by reducing the number of monocytes and macrophages as well as decreasing dermal fibronectin and collagen deposition in rats Shah M., *J. Cell. Sci.*, 1995, 108, 985-1002. Moreover, TGF-β antibodies also improve healing of corneal wounds in rabbits Moller-Pedersen T., *Curr. Eye Res.*, 1998, 17, 736-747, and accelerate wound healing of gastric ulcers in the rat, Ernst H., *Gut*, 1996, 39, 172-175. These data strongly suggest that limiting the activity of TGF-β would be beneficial in many tissues and suggest that any disease with chronic elevation of TGF-β would benefit by inhibiting smad2 and smad3 signalling pathways.

TGF-β is also implicated in peritoneal adhesions Saed G.M., *et al*, *Wound Repair Regeneration*, 1999 Nov-Dec, 7(6), 504-510. Therefore, inhibitors of ALK5 would be beneficial in preventing peritoneal and sub-dermal fibrotic adhesions following surgical procedures.

TGF-β is also implicated in photoaging of the skin (see Fisher GJ. Kang SW. Varani J. Bata-Csorgo Z. Wan YS. Data S. Voorhees JJ. , Mechanisms of photoaging and chronological skin ageing, *Archives of Dermatology*, 138(11):1462-1470, 2002 Nov. and Schwartz E. Sapadin AN. Kligman LH. "Ultraviolet B radiation increases steady state mRNA levels for cytokines and integrins in hairless mouse skin- modulation by topical tretinoin", Archives if Dermatological Research, 290(3):137-144, 1998 Mar.)

Therefore according to a further aspect, the invention provides the use of a compound defined in the first aspect in the preparation of a medicament for treating or preventing a disease or condition mediated by ALK-5 inhibition.

Preferably the disease or condition mediated by ALK-5 inhibition is selected from the list: chronic renal disease, acute renal disease, wound healing, arthritis, osteoporosis, kidney disease, congestive heart failure, ulcers (including diabetic ulcers, chronic ulcers, gastric ulcers, and duodenal ulcers), ocular disorders, corneal wounds, diabetic nephropathy, impaired neurological function, Alzheimer's disease, atherosclerosis, peritoneal and sub-dermal adhesion, any disease wherein fibrosis is a major component, including, but not limited to kidney fibrosis, lung fibrosis and liver fibrosis, for example, hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol-induced hepatitis, haemochromatosis, primary biliary cirrhosis, restenosis, retroperitoneal fibrosis, mesenteric fibrosis, endometriosis, keloids, cancer, abnormal bone function, inflammatory disorders, scarring and photaging of the skin.

More preferably the disease or condition mediated by ALK-5 inhibition is fibrosis. Preferably kidney fibrosis.

It will be appreciated that references herein to treatment extend to prophylaxis as well as the treatment of established conditions.

Compounds of the invention may be administered in combination with other therapeutic agents, for example antiviral agents for liver diseases, or in combination with ACE inhibitors or angiotensin II receptor antagonists for kidney diseases.

The compounds of the invention may be administered in conventional dosage forms prepared by combining a compound of the invention with standard pharmaceutical carriers or diluents according to conventional procedures well known in the art. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation.

The pharmaceutical compositions of the invention may be formulated for administration by any route, and include those in a form adapted for oral, topical or parenteral administration to mammals including humans.

The compositions may be formulated for administration by any route. The compositions may be in the form of tablets, capsules, powders, granules, lozenges, creams or liquid preparations, such as oral or sterile parenteral solutions or suspensions.

The topical formulations of the present invention may be presented as, for instance, ointments, creams or lotions, eye ointments and eye or ear drops, impregnated dressings and aerosols, and may contain appropriate conventional additives such as preservatives, solvents to assist drug penetration and emollients in ointments and creams.

The formulations may also contain compatible conventional carriers, such as cream or ointment bases and ethanol or oleyl alcohol for lotions. Such carriers may be present as from about 1% up to about 98% of the formulation. More usually they will form up to about 80% of the formulation.

Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; or acceptable wetting agents such as

sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives, such as suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats, emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl *p*-hydroxybenzoate or sorbic acid, and, if desired, conventional flavouring or colouring agents.

Suppositories will contain conventional suppository bases, e.g. cocoa-butter or other glyceride.

For parenteral administration, fluid unit dosage forms are prepared utilising the compound and a sterile vehicle, water being preferred. The compound, depending on the vehicle and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilised before filling into a suitable vial or ampoule and sealing.

Advantageously, agents such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. The dry lyophilised powder is then sealed in the vial and an accompanying vial of water for injection may be supplied to reconstitute the liquid prior to use. Parenteral suspensions are prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilisation cannot be accomplished by filtration. The compound can be sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

The compositions may contain from 0.1% by weight, preferably from 10-60% by weight, of the active material, depending on the method of administration. Where the

compositions comprise dosage units, each unit will preferably contain from 50-500 mg of the active ingredient. The dosage as employed for adult human treatment will preferably range from 100 to 3000 mg per day, for instance 1500 mg per day depending on the route and frequency of administration. Such a dosage corresponds to 1.5 to 50 mg/kg per day. Suitably the dosage is from 5 to 20 mg/kg per day.

It will be recognised by one of skill in the art that the optimal quantity and spacing of individual dosages of a compound of the invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular mammal being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of a compound of the invention given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

No toxicological effects are indicated when a compound of the invention is administered in the above-mentioned dosage range.

All publications, including, but not limited to, patents and patent applications cited in this specification, are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

It will be appreciated that the invention includes the following further aspects. The preferred embodiments described for the first aspect extend these further aspects:

i) a pharmaceutical composition comprising a compound of the invention and a pharmaceutically acceptable carrier or diluent;

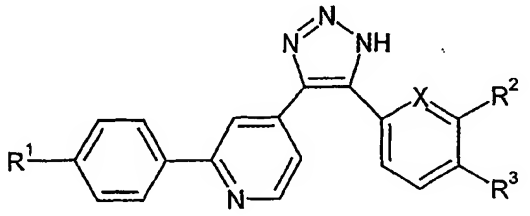
ii) a compound of the invention for use as a medicament;

iii) a method of treatment or prophylaxis of a disorder selected from chronic renal disease, acute renal disease, wound healing, arthritis, osteoporosis, kidney disease, congestive heart failure, ulcers (including diabetic ulcers, chronic ulcers, gastric ulcers, and duodenal ulcers), ocular disorders, corneal wounds, diabetic

nephropathy, impaired neurological function, Alzheimer's disease, atherosclerosis, peritoneal and sub-dermal adhesion, any disease wherein fibrosis is a major component, including, but not limited to kidney fibrosis, lung fibrosis and liver fibrosis, for example, hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol-induced hepatitis, haemochromatosis, primary biliary cirrhosis, restenosis, retroperitoneal fibrosis, mesenteric fibrosis, endometriosis, keloids, cancer, abnormal bone function, inflammatory disorders, scarring and photoaging of the skin, in mammals, which comprises administration to the mammal in need of such treatment, an effective amount of a compound of the invention; and

iv) a combination of a compound of the invention with an ACE inhibitor or an angiotensin II receptor antagonist.

According to a further aspect, the invention provides a compound of formula (I)



(I)

wherein X is N or CH;

R^1 is selected from H, C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkoxy, halo, cyano, perfluoro C_{1-6}alkyl, perfluoroC_{1-6}alkoxy, $-NR^4R^5$, $-(CH_2)_nR^4R^5$, $-O(CH_2)_nOR^6$, $-O(CH_2)_nNR^4R^5$, $-CONR^4R^5$, $-CO(CH_2)_nNR^4R^5$, $-SO_2R^6$, $-SO_2NR^4R^5$, $-NR^5SO_2R^6$ and $-NR^4COR^6$;

R^2 is selected from H, C_{1-6}alkyl, halo, CN or perfluoroC_{1-6}alkyl;

R^3 is selected from H or halo;

R^4, R^5 and R^6 are independently selected from H or C_{1-6}alkyl; or R^4 and R^5 together with the atom to which they are attached form a 3, 4, 5, 6 or 7-membered saturated or unsaturated ring which may contain one or more heteroatoms selected from N, S or O, and wherein the ring may be further substituted by one or more substituents selected from halo (such as fluoro, chloro, bromo), -CN, $-CF_3$, -OH, $-OCF_3$, C_{1-6} alkyl and C_{1-6} alkoxy; and

n is 1-4.

The following non-limiting examples illustrate the present invention.

Abbreviations

CuI	copper(I) iodide
CH_2Cl_2	dichlorometane
DME	1,2-dimethoxyethane
DMF	dimethylformamide
EtOAc	ethyl acetate
K_2CO_3	potassium carbonate
MeOH	methanol
Na_2CO_3	sodium carbonate
$NaNO_2$	sodium nitrite
NaOH	sodium hydroxide
Na_2SO_4	sodium sulfate
NH_4Cl	ammonium chloride
$Pd(PPh_3)_4$	tetrakis(triphenlyphosphine) palladium(0)
TMS	trimethylsilyl
$TMSN_3$	trimethylsilyl azide
THF	tetrahydrofuran
TMEDA	N,N,N',N'- Tetramethylethylenediamine

Intermediate 1: 2-Chloro-4-iodo-pyridine

To a ice-cooled solution of 4-amino-2-chloro-pyridine (8.09g, 63 mmol, 1eq) in water (150mL) was added concentrated 98% HCl whilst maintaining the reaction at 0°C. A solution of sodium nitrite (5.65g, 82mmol, 1.3eq) in water (50mL) was added slowly at –10°C. The mixture was stirred at –10°C for 40 min and a solution of potassium iodide (12.55g, 75.6mmol, 1.2eq) in water (50mL) was added. The resulting mixture was stirred at 0°C overnight. After treatment with NaOH 35%, and extraction with ethyl acetate, the organic phases were combined and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel (eluent: CH_2Cl_2 then CH_2Cl_2/ CH_3OH 99/1) to give the title compound as an orange solid (9.5g, 63%); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.99 (d, 1H), 7.68 (s, 1H), 7.52 (d, 1H); (GC-MS) m/z: 239.

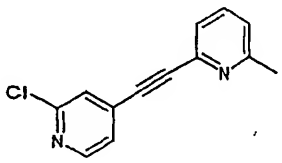
Intermediate 2 : 2-Methyl-6-trimethylsilanylethynyl-pyridine

To a solution of 2-bromo-4-methyl-pyridine (25g, 0.15 mol) in dry THF (200mL), were added TMEDA (200mL) and TMS-acetylene (100mL, excess) under N_2. The resulting mixture was degassed with nitrogen for 10 min, then tetrakis(triphenlyphosphine) palladium(0) (3.7mmol, 4.3g) and copper iodide (14.7mmol, 2.8g) were added. The resulting mixture was heated at 60°C for 18h. The reaction mixture was concentrated and the residue partitioned between ethyl acetate / water. The organic phase was dried over Na_2SO_4 and filtered. Evaporation of the solvent *in vacuo* gave a crude product which was purified by chromatography on silica gel (CH_2Cl_2) to give the title compound (18.4g, 65%) as a black oil; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.58-7.49 (m, 1H), 7.30 (d, 1H), 7.10 (d, 1H), 2.56 (s, 3H), 0.28 (s, 9H).

Intermediate 3 : 2-Ethynyl-6-methyl-pyridine

To a solution of Intermediate 2 (18.4g, 0.097mol) in MeOH (100 ml) was added potassium carbonate (4eq, 0.39mol, 53.7g). The reaction mixture was then stirred at room temperature for 30 min and the solvent evaporated to dryness. The residue was partitioned between ethyl acetate / water. The organic layer was dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure to give the title compound (8.75g, 77%) as a brown oil; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.45-7.34 (m, 1H), 7.14 (d, 1H), 6.98 (d, 1H), 2.97 (s, 1H), 2.40 (s, 3H).

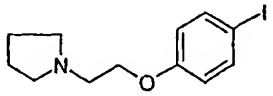
Intermediate 4 : 6-Methyl-2-[(2-chloro-pyridin-4-yl)-ethynyl]-pyridine



To a solution of intermediate 1 (1.85g, 7.74mmol) in dry THF (40mL) were added under nitrogen, TMEDA (20mL) and intermediate 3 (1.1eq, 1g, 8.51mmol). The resulting mixture was degassed with nitrogen for 10 min, then tetrakis(triphenlyphosphine) palladium(0) (0.464mmol, 537mg) and copper iodide (0.928 mmol, 177mg) were added. The resulting mixture was heated at 60°C for 4h. The mixture was poured into a saturated solution of NH_4Cl and extracted with EtOAc. The organic phase was dried over Na_2SO_4 and filtered. Solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel (CH_2Cl_2/EtOAc 90:10) to afford the title compound as a beige solid (1.54g, 86.4%);

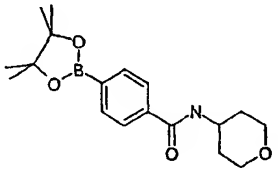
^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.29 (d, 1H), 7.52 (t, 1H), 7.39 (s, 1H), 7.34-7.24 (m, 2H), 7.10 (d, 1H), 2.50 (s, 3H).

Intermediate 5 : 4-(2-(pyrrolidin-1-yl)-ethoxy)-iodobenzene



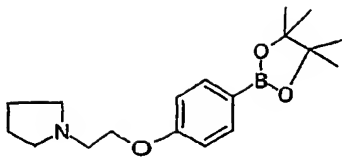
To a solution of 4-iodo-phenol (6g, 27.3mmol) in acetone (200ml) were added cesium carbonate (22.2g, 68.4 mmol) and N-(2-chloroethyl)-pyrrolidine.hydrochloride (7g, 41 mmol) and the mixture was heated under reflux for 4 h and then poured into water. After extraction with CH_2Cl_2, the organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The title compound was obtained as a red oil (8g, 92.53%); ^{1}H NMR (300MHz, $CDCl_3$) δ ppm: 7.5 (d, 2H), 6.65 (d, 2H), 4 (t, 2H), 2.8 (t, 2H), 2.55 (m, 4H), 1.75 (m, 4H).

Intermediate 6: 4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-*N*-(tetrahydro-pyran-4-yl)-benzamide



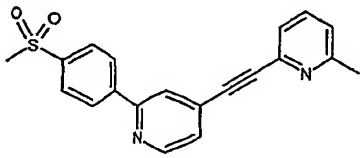
4-(4,4,5,5-Tetramethyl-[1,3,2]dioxaborolan-2-yl)-benzoic acid (70.16g, 0.28 mol) was treated with $SOCl_2$ (2 vol.) and the reaction mixture was stirred at reflux for 2 hours. After evaporation, the residue was diluted with toluene and poured into a solution at 10°C of tetrahydro-pyran-4-ylamine (34.34g, 0.339) and triethylamine (79 mL, 0.57 mol) in CH_2Cl_2. The reaction mixture was stirred at room temperature for 2 days and water (490 mL) was added to give a precipitate which was filtered and washed with EtOAc. After purification by flash chromatography using CH_2Cl_2/MeOH (95:5), the title compound was obtained as a solid (17.02g, 18%); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.85 (d, 2H), 7.72 (d, 2H), 5.98 (m, 1H), 4.20 (s, 1H), 3.99 (m, 2H), 3.35 (t, 2H), 2.01 (d, 2H), 1.57 (m, 2H), 1.35 (s, 12H).

Intermediate 7: 1-[2-(pyrrolidin-1-yl)-ethoxy]-4-[4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl]-benzene



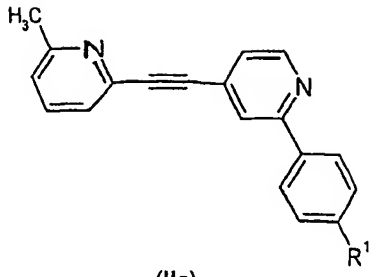
To a solution of intermediate 5 (8g, 25.24mmol) in dioxane (200ml) was added 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (4ml, 27.6mmol), dichlorobis (triphenylphosphine)palladium(II) (0.88g, 1.26mmol), triethylamine (10.5ml, 75.72mmol) and the mixture was heated under reflux for 4 hours and then poured into water. After extraction with CH_2Cl_2, the organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (9:1). The titled compound was obtained as a yellow solid (8g, 99.99%); mp : 160-164°C.

Intermediate 8: 2-[(2-(4-methylsulfonylphenyl)-pyridin-4-yl)-ethynyl]-6-methyl-pyridine



Intermediate 4 (1g, 4.37mmol) and 4-(methylsulfonyl)phenyl boronic acid (1.14g, 5.7 mmol), were dissolved in a mixture of toluene (30mL) and EtOH (10mL). To this solution were added tetrakis(triphenlyphosphine) palladium(0) (0.118 g, 0.1mmol) and aqueous sodium carbonate 2M (8.6mL, 17.2mmol) under nitrogen. The resulting mixture was stirred under reflux for 6 h. The mixture was hydrolysed with water and extracted with ethyl acetate, the combined organic phases were washed with water and dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give a crude product which was purified by chromatography on silica gel (eluent : CH_2Cl_2/CH_3OH 98:2) to give the title compound as a yellow oil (0.7g, 46%); ^1H NMR (300 MHz, $CDCl_3$) δ ppm: 8.66 (d, 1H), 8.14 (d, 2H), 7.98 (d, 2H), 7.90 (s, 1H), 7.56 (t, 1H), 7.43-7.32 (m, 2H), 7.12 (d, 1H), 3.03 (s, 3H), 2.50 (s, 3H); [APCI MS] m/z 349 (MH^+).

Compounds of formula (IIc), i.e. compounds of formula (II) where X is N, R^2 is methyl and R^3 is hydrogen (see Table 1), were prepared by methods analogous to those described for intermediate 8 using the appropriate boronic acid derivatives.

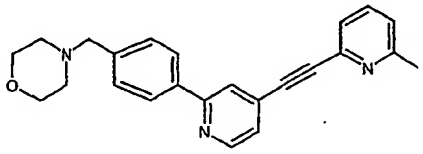


(IIc)

Table 1

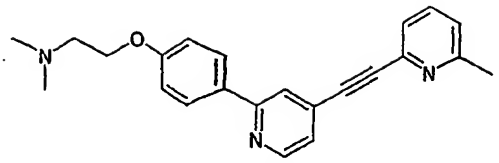
Int.	R^1	Physical data
9	methoxy	APCI MS m/z 301 (MH+)
10	tetrahydropyran-4-ylaminocarbonyl	APCI MS m/z 398 (MH+)
11	hydroxy	APCI MS m/z 287 (MH+)
12	ethyl	APCI MS m/z299 (MH+)
13	chloro	APCI MS m/z 306 (MH+)
14	trifluoromethoxy	APCI MS m/z 355 (MH+)
15	2-(pyrrolidino)ethoxy	APCI MS m/z 384 (MH+)
16	fluoro	APCI MS m/z 289 (MH+)
17	formyl	APCI MS m/z 299 (MH+)

Intermediate 18: 4-{4-[4-(6-Methyl-pyridin-2-ylethynyl)-pyridin-2-yl]-benzyl}-morpholine



To a solution of intermediate 17 (0.45g, 1.5mmol) in dichloroethane (40ml) were added morpholine (0.9g, 6mmol), sodium triacetoxyborohydride (1.2g, 6mmol) and acetic acid (0.27g, 4mmol). The mixture was stirred at room temperature for 6 hours and then poured into ice and extracted with CH_2Cl_2 . The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give the title compound as a yellow oil (0.55g, quantitative); [APCI MS] m/z= 370 (MH+).

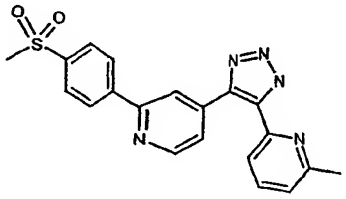
Intermediate 19 : *N,N*-dimethyl-2-[(4-{4-(6-methyl-pyridin-2-yl-ethynyl)-pyridin-2-yl}phenyl)oxy]ethanamine



To a solution of intermediate 11 (0.572g, 2mmol) in acetone (20ml) were added 2-chloro-*N*,*N*-dimethylethanamine hydrochloride (0.374g, 2.6mmol) and potassium carbonate (0.822g, 6mmol). The mixture was stirred under reflux overnight. The reaction mixture was filtered and concentrated under reduced pressure. The residue was poured into water and extracted with CH_2Cl_2 . The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give the title compound as a brown oil (0.7g, 98%); [APCI MS] m/z= 358 (MH+).

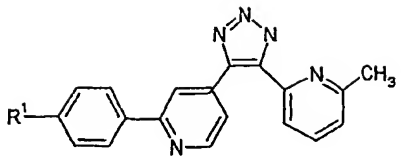
Examples

Example 1 : 2-(4-Methanesulfonylphenyl)-4-(5-(6-methyl)-pyridin-2-yl-3H-[1,2,3]triazol-4-yl)-pyridine



To a solution of Intermediate 8 (700mg, 2 mmol) in dry DMF (13 ml) was added azidotrimethylsilane (8 mmol, 930mg). The reaction mixture was then stirred at 100°C overnight. The reaction mixture was hydrolysed with water and extracted with CH_2Cl_2 . The organic phase was washed with water, dried over Na_2SO_4 and filtered. Evaporation of the solvent *in vacuo* gave a crude product which was purified by chromatography on silica gel (toluene / isopropylamine 95:5). The crude oil was precipitated in a mixture CH_2Cl_2/hexane to give the title compound as a yellow powder (260mg, 33.2%), gummy at 150°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.70 (d, 1H,), 8.28 (s, 1H), 8.15 (d, 2H), 7.95 (d, 2H), 7.70-7.57 (m, 2H, 7.50 (d, 1H), 7.15 (d, 1H), 3.00 (s, 3H), 2.50 (s, 3H), NH triazole not observed; TOF MS ES$^+$ exact mass calculated for $C_{20}H_{17}N_5O_2S$: 392.1181 (MH+). Found : 392.1218 (MH+).

Compounds of formula (Ia), i.e. compounds of formula (I) where X is N, R^2 is methyl and R^3 is hydrogen (see Table 2), were prepared by methods analogous to that described for Example 1 from the intermediate indicated in the table.



(Ia)

Table 2

Ex	R^1	From intermediate:	Physical data
2	methoxy	9	TOF MS ES$^+$ exact mass calculated for $C_{20}H_{17}N_5O$: 344.1511(MH+). Found: 344.1506(MH+).
3	2-(N,N-dimethylamino)ethoxy	19	TOF MS ES$^+$ exact mass calculated for $C_{23}H_{24}N_6O$: 401.2090(MH+). Found:401.2063(MH+). m.p. 143°C
4	morpholinomethyl	18	TOF MS ES$^+$ exact mass calculated for $C_{24}H_{24}N_6O$: 413.2090 (MH+) . Found: 413.2110 (MH+). m.p. 110°C
5	ethyl	12	APCI MS m/z 342 (M+1)
6	tetrahydropyran-4-ylaminocarbonyl	10	TOF MS ES$^+$ exact mass calculated for $C_{25}H_{24}N_6O$: 441.2039(MH+). Found: 441.2032(MH+).
7	chloro	13	TOF MS ES$^+$ exact mass calculated for $C_{19}H_{14}ClN_5$: 348.1016 (MH+) Found:348.1000 (MH+); m.p. 144°C
8	trifluoromethoxy	14	TOF MS ES$^+$ exact mass calculated for $C_{20}H_{14}F_3N_5O$: 398.1229 (MH+). Found: 398.1194; m.p. 128°C
9	2-(pyrolidin-1-yl)ethoxy	15	TOF MS ES$^+$ exact mass calculated for $C_{25}H_{26}N_6O$: 427.2246 (MH+). Found: 427.2277(MH+).
10	fluoro	17	TOF MS ES$^+$ exact mass calculated for $C_{19}H_{14}FN_5$: 332.1311 (MH+). Found: 332.1345 (MH+).

Biology

The biological activity of the compounds of the invention may be assessed using the following assays:

Assay 1 (Cellular transcriptional assay)

The potential for compounds of the invention to inhibit TGF-β signalling may be demonstrated, for example, using the following *in vitro* assay.

The assay was performed in HepG2 cells stably transfected with the PAI-1 promoter (known to be a strong TGF-β responsive promoter) linked to a luciferase (firefly)

reporter gene. The compounds were selected on their ability to inhibit luciferase activity in cells exposed to TGF-β. In addition, cells were transfected with a second luciferase (Renilla) gene which was not driven by a TGF-β responsive promoter and was used as a toxicity control.

96 well microplates were seeded, using a multidrop apparatus, with the stably transfected cell line at a concentration of 35000 cells per well in 200 µl of serum-containing medium. These plates were placed in a cell incubator.

18 to 24 hours later (Day 2), cell-incubation procedure was launched. Cells were incubated with TGF-β and a candidate compound at concentrations in the range 50 nM to 10 µM (final concentration of DMSO 1%). The final concentration of TGF-β (rhTGFβ-1) used in the test was 1 ng/mL. Cells were incubated with a candidate compound 15-30 mins prior to the addition of TGF-β. The final volume of the test reaction was 150 µl. Each well contained only one candidate compound and its effect on the PAI-1 promoter was monitored.

Columns 11 and 12 were employed as controls. Column 11 contained 8 wells in which the cells were incubated in the presence of TGF-β, *without* a candidate compound. Column 11 was used to determine the 'reference TGF-β induced firefly luciferase value' against which values measured in the test wells (to quantify inhibitory activity) were compared. In wells A12 to D12, cells were grown in medium without TGF-β. The firefly luciferase values obtained from these positions are representative of the 'basal firefly luciferase activity'. In wells E12 to H12, cells were incubated in the presence of TGF-β and 500 µM CPO (Cyclopentenone, Sigma), a cell toxic compound. The toxicity was revealed by decreased firefly and renilla luciferase activities (around 50 % of those obtained in column 11).

12 to 18 hours later (day 3), the luciferase quantification procedure was launched. The following reactions were performed using reagents obtained from a Dual Luciferase Assay Kit (Promega). Cells were washed and lysed with the addition of 10 µl of passive lysis buffer (Promega). Following agitation (15 to 30 mins), luciferase activities of the plates were read in a dual-injector luminometer (BMG lumistar). For this purpose, 50 µl of luciferase assay reagent and 50 µl of 'Stop & Glo' buffer were injected sequentially to quantify the activities of both luciferases. Data obtained from the measurements were processed and analysed using suitable software. The mean Luciferase activity value obtained in wells A11 to H11 (Column 11, TGF-βonly) was

considered to represent 100% and values obtained in wells A12 to D12 (cells in medium alone) gave a basal level (0%). For each of the compounds tested, a concentration response curve was constructed from which an IC_{50} value was determined graphically.

Assay 2 (Alk5 Fluorescence Polarization Assay)

Kinase inhibitor compounds conjugated to fluorophores, can be used as fluorescent ligands to monitor ATP competitive binding of other compounds to a given kinase. The increase in depolarization of plane polarized light, caused by release of the bound ligand into solution, is measured as a polarization/anisotropy value. This protocol details the use of a rhodamine green-labelled ligand for assays using recombinant GST-ALK5 (residues 198-503).

Assay buffer components: 62.5 mM Hepes pH 7.5 (Sigma H-4034), 1 mM DTT (Sigma D-0632), 12.5 mM $MgCl_2$ (Sigma M-9272), 1.25 mM CHAPS (Sigma C-3023).

Protocol: Solid compound stocks were dissolved in 100% DMSO to a concentration of 1 mM and transferred into column 1, rows A-H of a 96-well, U bottom, polypropylene plate (Costar #3365) to make a compound plate. The compounds were serially diluted (3-fold in 100% DMSO) across the plate to column 11 to yield 11 concentrations for each test compound. Column 12 contained only DMSO. A Rapidplate™-96 was used to transfer 1 µl of sample from each well into a 96-well, black, U-bottom, non-treated plate (Costar #3792) to create an assay plate.

ALK5 was added to assay buffer containing the above components and 1 nM of the rhodamine green-labelled ligand so that the final ALK5 concentration was 10 nM based on active site titration of the enzyme. The enzyme/ligand reagent (39 µl) was added to each well of the previously prepared assay plates. A control compound (1 µl) was added to column 12, rows E-H for the low control values. The plates were read immediately on a LJL Acquest fluorescence reader (Molecular Devices, serial number AQ1048) with excitation, emission, and dichroic filters of 485nm, 530 nm, and 505 nm, respectively. The fluorescence polarization for each well was calculated by the Acquest reader and then imported into curve fitting software for construction of concentration response curves. The normalized response was determined relative to the high controls (1 µl DMSO in column 12, rows A-D) and the

low controls (1 µl of control compound in column 12, rows E-H). An IC_{50} value was then calculated for each compound

Using the above assays all Examples of the invention show ALK5 receptor modulator activity (having IC_{50} values in the range of 1 to 200nM) and TGF-β cellular activity (having IC_{50} values in the range of 0.001 to 10µM).

2-(4-Methanesulfonylphenyl)-4-(5-(6-methyl)-pyridin-2-yl-3H-[1,2,3]triazol-4-yl)-pyridine (Example 1) showed an ALK5 receptor modulator activity of 26 nM and TGF-β cellular activity of 413 nM.